C3

41. **(Amended)** The prokaryotic cell of claim 40, wherein the further modification is a mutation in the *ahpC* gene which reduces its peroxidase activity.

C4

43. **(Amended)** The prokaryotic cell of claim 42, wherein the mutation results in a mutated AhpC protein that has the amino acid sequence set forth in SEQ ID NO: 24.

C5

46. **(Amended)** The prokaryotic cell of claim 44, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its wild-type counterpart.

C6

50. **(Amended)** A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 29 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.

Remarks

Claims 1-50, 55 and 56 are pending in the application. Claims 29-33, 39-41, 43, 46 and 50 have been amended. Support for the amendments may be found throughout the specification, including the claims as originally filed. No new matter has been added.

Amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite prosecution of the present application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Applicants respectfully request entry of these claim amendments, since these amendments present the claims in better form for consideration on appeal.

Claim Objections

The Examiner objected to claims 30, 32 and 46 because these claims all use the phrase "wild type" as an adjective without hyphenating the phrase. Claims 30, 32 and 46 have been amended to include a hyphen in the phrase "wild type." The claim amendment is believed to obviate the objection. Reconsideration and withdrawal of the objection is respectfully requested.

The Examiner objected to claim 50 because of a grammatical error. Claim 50 has been amended as suggested by the Examiner. The claim amendment is believed to obviate the objection. Reconsideration and withdrawal of the objection is respectfully requested.

Rejection of claims 29-42, 44-48 and 50 under 35 U.S.C 112, first paragraph

The Examiner states that claims 29-42, 44-48 and 50 stand rejected under 35 U.S.C. 112, first paragraph because, according to the Examiner, they describe subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the invention at the time the application was filed. The rejection is respectfully traversed.

The Examiner acknowledges that an Applicant "may show that he is in "possession" of the invention claimed by describing the invention with all of its claimed limitations "by such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention." See *Lockwood v. American Airlines Inc.* 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997)." Applicants respectfully submit that the specification does describe the invention with all of its claimed limitations in a sufficiently comprehensive manner as to convey to one skilled in the art that the inventors were in possession of the invention claimed in claims 29-42, 44-48 and 50 at the time of filing.

The Examiner objects to claim 29 as that claim is "a genus claim in terms of any genetic modification that increases the cell's ability to proliferate." According to the Examiner, "the specification mentions a specific mutation in the AhpC" and the "disclosure is not deemed descriptive of the complete structure of a representative number of species encompassed by the claims as one skilled in the art cannot envision all genetic modifications that will increase the ability to proliferate based on the teachings in the specification." In particular, the Examiner argues that "the specification fails to teach any other specific examples of genetic modifications which increase proliferation. No other specific genes are suggested for mutation, suppressor or otherwise, or for heterologous expression to achieve the proliferation increase."

Applicants respectfully point out that, as the Examiner himself acknowledges, the specification does contain teachings "that the genetic mutation can involve mutation of a host gene or by the introduction of a gene into the host cell (pp. 12-13)." Based on additional teachings contained in the disclosure and methodologies that are well known in the field, one

skilled in the art could readily identify either mutations in the host gene, or mutations in a gene that is later introduced into the host cell, that increase the cell's ability to proliferate.

The specification teaches an assay method for identifying mutations that increase the proliferation rate of prokaryotic cells that are already genetically modified to shift the redox status of the cytoplasm to a more oxidative state that favors disulfide bond formation. The Examiner himself acknowledges that "the specification teaches how to screen for such genetic modifications (pp 18-20)." Briefly, according to the specification, one could screen for such mutations by "growing cells in the presence of DTT for a certain time period, removing DTT from the culture media, and selecting fast growing colonies." (pp 18, lines 30-31). One skilled in the art would readily be able to utilize the assay system disclosed to identify additional mutations, either in the AhpC gene or in other genes, that increase a prokaryotic cell's ability to proliferate. The application further teaches methods to characterize novel suppressor mutations identified by the assay method described above. Specifically, the specification discusses the use of prokaryotic genetics, e.g. linkage analysis, to determine the identity of a novel suppressor mutant (pp. 19). Furthermore, the specification discloses another class of enzymes, the ribonucleotide reductases, as good candidates for alternative suppressor mutations (p 20).

Examiner further argues that "the single species disclosed in the specification was inadvertently identified (p. 42, Example 1) and characterized as a suppressor mutation." Applicants respectfully disagree. The species disclosed in the application, the AhpC mutant, was identified and characterized using the methodology described in the specifications (pp 18-20) and, based on that method, the mutation was determined to restore the ability of the cell to proliferate at a normal rate. The mutant's ability to restore a more normal rate of proliferation, which was lost as a result of the primary genetic modification, qualifies it as a suppressor mutation.

The Examiner states that for "the single species disclosed, a specific mutation of the AhpC protein, there is no structure-function correlation taught indicating where other mutations to AhpC would yield the desired phenotype. There is no teaching of what amino acids are in the hydrophobic core of the protein, in the catalytic site or in the binding pocket which would demonstrate the Applicant knew where to make genetic mutations to reduce its peroxidase activity." Applicants respectfully point out that based on the teachings in the specification one skilled in the art would be able to screen for other mutants in the AhpC protein that alter the

redox potential of the cytoplasm sufficiently to increase the proliferation rate of a prokaryotic cell that is already genetically modified to shift the redox status of the cytoplasm to a more oxidative state that favors disulfide bond formation. As discussed above, the specification discloses a simple assay that allows one skilled in the art to screen and identify genetic mutants that increase the proliferation rate of a prokaryotic cell. One skilled in the art would then be able to characterize the suppressor mutant based on methodologies standard in the art of prokaryotic genetics (p19). Alternatively, other mutations in, e.g., AhpC or other genes, can be identified by introducing random mutations into prokaryotic cells that are already genetically modified to shift the redox status of the cytoplasm to a more oxidative state that favors disulfide bond formation, and selecting those cells which show an increased growth rate (pp 19-20).

Additionally, the sequence of the AhpC protein is known and one skilled in the art would be able to determine from the sequence which regions of the gene would be more likely to increase the catalytic activity of the protein. Additionally, based on the screening methods disclosed in the specification, one skilled in the art could readily identify certain residues or regions of the protein that are important in modulating the bioactivity of either AhpC or another gene of interest. In particular, the specification discloses multiple methods that can be used to identify other mutants that would alter the catalytic activity of a protein, e.g. AhpC (pp 25-30). For example, one skilled in the art could identify additional variants with the use of alanine scanning mutagenisis or random mutagenesis (p 26).

Accordingly, a person of skill in the art would reasonably conclude that Applicants were in possession of the claimed invention at the time the application was filed. Therefore, reconsideration and withdrawal of the rejection is respectfully requested.

The Examiner objects to claim 46 because it is drawn to a prokaryotic cell having a catalyst of disulfide formation and/or isomerization wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its wild-type counterpart. The Examiner argues that, while the specification does disclose "mutations in the -C-Xaa-Xaa-C- active site of most cysteine oxidoreductases (p. 23)," it is "not deemed to be descriptive of the complete structure of a representative number of species encompassed by the claims as one skilled in the art cannot envision all the variants that will increase the redox potential of any of the thioredoxin superfamily of proteins based on the teachings in the

specification." Furthermore, the Examiner argues that, while the disclosure "teaches that variants may include amino acid substitutions, deletions or additions (p.23, lines 12-13). There is no disclosure, other than of the active site motif -C-Xaa-Xaa-C-, of regions where substitutions, deletions or additions would yield increased redox potential." Applicants respectfully traverse the rejection.

Applicants respectfully point out that the specification discloses multiple methods of identifying thioredoxin variants having a redox potential that is higher than that of its wild-type counterpart (pp 22-30). In particular, the specification contemplates the identification of thioredoxin variants from other species or genuses by hybridization techniques or with the use of cross-reacting antibodies as it is well known in the art that catalysts of disulfide bond formation and/or isomerization are relatively well conserved (p 25). Additionally, the design of variants may be facilitated by aligning the sequences of the members of thioredoxin superfamily (p 25). Additionally, variants of the thioredoxin family may be identified by in vivo genetic screens (p 25), with the use of alanine scanning mutagenesis (p 26), or random mutagenesis (p 26). Furthermore, the specification discloses methods of determining the redox potential of a protein, (pp 23-24), which would allow one skilled in the art to assess the effectiveness of the thioredoxin variant as a catalyst of disulfide bond formation.

Therefore, Applicants respectfully submit that the written description discloses a representative number of species of the claimed genus. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of claims 55 and 56 under 35 U.S.C 112, first paragraph

The Examiner objects to claims 55 and 56 under 35 U.S.C. §112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants submit that a deposit is not necessary to satisfy the requirements of 35 U.S.C. §112, however, in an effort to expedite prosecution of this application, Applicants have amended the specification as requested by the examiner to insert the date of the depository. Additionally, Applicants are submitting concurrently herewith a Declaration regarding the deposits having ATCC Accession Numbers PTA-938 (FA112) and PTA-939 (FA113). Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of claims 29-48, 50, 55 and 56 under 35 U.S.C. 112, second paragraph

Claims 29-48, 50, 55 and 56 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

Claim 29 was rejected for reciting two different genetic modifications without clarifying which is the first modification and which is the second modification. Additionally, the claim recites "a more oxidative state that favors disulfide bond formation" without clarifying to what the cytoplasm has a relatively more oxidative state. Finally, claim 29 recites "contains a genetic modification that increases it ability to proliferate" without clarifying relative to what the ability to proliferate is increased. Claim 29 has been amended as suggested by the Examiner. The claim amendments are believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 31 was rejected for reciting "reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase and glutathione" and the Examiner notes that "glutathione" is not a reductase. Claim 31 has been amended to delete the "glutathione" from the group. The claim amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 33 was rejected for reciting "reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase and glutathione" and the Examiner notes that "glutathione" is not a reductase. Claim 33 has been amended to delete the "glutathione" from the group. The claim amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 39 was rejected for reciting "the genetic modification" without clarifying to which of the two genetic modifications recited in parent claim 29 this refers. Claim 39 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 40 was rejected for reciting "modification" without clarifying to which of the two genetic modifications recited in parent claim 29 this refers. Claim 40 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 40 was rejected for reciting "restores at least some of the reducing capacity" without clarifying relative to what the reducing capacity is restored. Claim 40 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 41 was rejected for reciting "the modification" without clarifying to which of the two genetic modifications recited in parent claim 29 this refers. Claim 41 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 43 was rejected for reciting the limitation "the mutated AhpC protein" in line 1 without a sufficient antecedent basis for the limitation. Claim 43 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

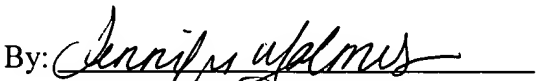
Claim 50 was rejected for reciting the limitation "a host cell of claim 29" in lines 1-2 without a sufficient antecedent basis for the limitation. Claim 50 has been amended and the amendment is believed to obviate the rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,

Customer No. 25181

By: 
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Dated: January 29, 2003

Copy of amendments to the specification with changes marked thereon

Please replace the paragraph beginning at **line 30** on **page 42** with the paragraph as set forth below. A marked-up version of the paragraph showing the changes made follows the remarks section of this response.

Two fast growing suppressor mutants were obtained from the strains DHB4 *gshA20*::Tn10Km *trxB*::Km...Tn10 and DHB4 *gor522*...mini-Tn10Tc *trxB*::Km, both of which are derivatives of DHB4 (MC1000 phoA(PvuII) phoR malF3 F'[lac+(lacIQ) pro]) (Boyd, et al. (1987) *Proc Natl Acad Sci U S A* 84: 8525), as follows. These fast growing suppressor mutants were obtained by growing the two strains for about 24 hours in medium containing 6 mM DTT. A fast growing strain from each of the two strains were isolated: FA112 ((DHB4 *gshA20*::Tn10Km *trxB*::Km...Tn10 *supp)* and FA113 (DHB4 *gor522*...mini-Tn10Tc *trxB*::Km *supp*). Each of these strains was deposited [at the ATCC] with the American Type Culture Collection (ATCC), 10801 University Blvd, Manassas, VA 20110-2209, on November 11, 1999, under the requirements and terms of the Budapest Treaty, and have been assigned Accession Nos. PTA-938 (FA112) and PTA-939 (FA113), respectively.

Copy of amended claims with changes marked thereon

Please amend claims 29-33, 39-41, 43, 46 and 50 as set forth below:

29. **(Amended)** A prokaryotic cell that is genetically modified to shift the redox status of the cytoplasm to a more oxidative state that favors disulfide bond formation, relative to wild-type, [and which further contains a genetic modification that increases its ability to proliferate] which cell is further genetically modified to increase the cell's ability to proliferate relative to cell that is not further genetically modified.

30. **(Amended)** The prokaryotic cell of claim 29, in which the expression or activity of a reductase is decreased relative to that in the corresponding [wild type] wild-type cell.

31. **(Amended)** The prokaryotic cell of claim 30, wherein the reductase is selected from the group consisting of thioredoxin reductase[,] and glutathione reductase[, and glutathione].

32. **(Amended)** The prokaryotic cell of claim 30, in which the expression or activity of a second reductase is decreased relative to that in the corresponding [wild type] wild-type cell.

33. **(Amended)** The prokaryotic cell of claim 29, wherein the second reductase is selected from the group consisting of thioredoxin reductase[,] and glutathione reductase[, and glutathione].

39. **(Amended)** The prokaryotic cell of claim 29, wherein the further genetic modification is a suppressor mutation.

40. **(Amended)** The prokaryotic cell of claim 29, wherein the further modification restores at least some of the reducing capacity to the cytoplasm of the prokaryotic cell relative to cell that is not further genetically modified.

41. **(Amended)** The prokaryotic cell of claim 40, wherein the further modification is a mutation in the *ahpC* gene which reduces its peroxidase activity.

43. **(Amended)** The prokaryotic cell of claim 42, wherein the mutation results in a mutated [ahpC] AhpC protein that has the amino acid sequence set forth in SEQ ID NO: 24.

46. **(Amended)** The prokaryotic cell of claim 44, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its [wild type] wild-type counterpart.

50. **(Amended)** A method for producing a protein having at least one disulfide bond[,] comprising: growing a [host] prokaryotic cell of claim 29 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.